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Reproducibility of HERMES-measured GABA+ and glutathione in the mesial temporal lobe

Marilena M. DeMayo [a,b,c,d,e,*], Mary Botros [a,c,d,f], Tiffany K. Bell [a,c,d], Mark Mikkelsen [g], Victoria Mosher [c], Antis George [c], Alexander McGirr [b,c,e], Paolo Federico [c], Ashley D. Harris [a,c,d]

[a] Department of Radiology, University of Calgary, Canada
[b] Department of Psychiatry, University of Calgary, Canada
[c] Hotchkiss Brain Institute, University of Calgary, Canada
[d] Alberta Children's Hospital Research Institute, University of Calgary, Canada
[e] Mathison Centre for Mental Health Research and Education, Canada
[f] School of Medicine, University of Galway, Canada
[g] Department of Radiology, Weill Cornell Medicine, Canada

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ABSTRACT

Background: There is growing interest in using Hadamard Encoding and Reconstruction for MEGA-Edited Spectroscopy (HERMES) within the mesial temporal lobe (MTL). For cross-sectional group comparisons and longitudinal repeated measures designs, an understanding of the internal and test-retest validity of γ-aminobutyric acid (GABA+) and glutathione (GSH) is critical. We therefore evaluated the reproducibility of the consensus recommended semi-localization by adiabatic selective refocusing (sLASER) localization for edited-MRS acquisitions in a challenging region, the MTL.

New method: Data were acquired in 15 participants. Single voxel HERMES was collected in the left MTL (two acquisitions) and the right MTL (one acquisition). Participants were repositioned between the two left HERMES acquisitions. An ANOVA was used to assess differences between acquisitions. To assess measurement variation in the repeated left of GABA+ and GSH measures within the left MTL difference values and coefficients of variation (CVs) were calculated.

Results: There were no significant differences in metabolite values between any of the acquisitions. The mean difference between the metabolite measures from the repeated left acquisitions centred close to zero, and the average CVs were 14.09 % for GABA+ and 18.94 % for GSH.

Comparison with existing methods: The CVs of GABA+ and GSH in the MTL obtained from a HERMES acquisition were comparable to GABA+ or GSH-edited acquisitions in this region, and to data from cortical voxels using HERMES acquisitions.

Conclusions: This supports the use of HERMES in the MTL, a challenging region for MRS. However, larger samples and caution in interpretation may be required in repeated-measures designs.

1. Introduction

There is great interest in acquiring magnetic resonance spectroscopy (MRS) data from the mesial temporal lobe (MTL), where the hippocampus is located. The hippocampus plays a key role in memory formation and retrieval (Knierim, 2015) and is impacted in neurodegenerative disorders, such as Alzheimer's Disease (Josephs et al., 2017) and in neurological disorders such as epilepsy (Thom, 2014). Acquiring high-quality MRS data from this region is particularly challenging, however, due to its proximity to sinuses and the resulting inhomogeneous B_0 field.

These challenges are amplified when performing edited MRS experiments, for instance, focusing on γ-aminobutyric acid (GABA), the brain's primary inhibitory neurotransmitter, and glutathione (GSH), a marker of oxidative stress. The multiplexed editing scheme, Hadamard Encoding and Reconstruction for MEGA-Edited Spectroscopy

* Correspondence to: 3280 Hospital Drive NW, Calgary, AB T2N 4Z6, Canada
E-mail address: marilena.demayo@ucalgary.ca (M.M. DeMayo).

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(HERMES), allows for the simultaneous measurement of metabolites that require editing, such as GABA and GSH (Chan et al., 2016; Saleh et al., 2016). This effectively halves the acquisition time needed to acquire both GABA- and GSH-edited MRS data, as two separate acquisitions are not necessary. Editing for low-signal metabolites, such as GABA and GSH, typically requires large voxels (Peek et al., 2023) and so when these metabolites are of interest in a small region such as the hippocampus, the prescribed voxel encompasses both the hippocampus and the surrounding tissue, or the MTL in this instance. Voxels will therefore quantify metabolites from this larger volume, which includes the hippocampus, amygdala, and entorhinal cortex (Van Hoesen, 1995) in the case of the MTL. For single voxel MRS, semi-localization by adiabatic selective refocusing (sLASER) localization has been recommended (Choi et al., 2021) due to the substantial reduction in chemical shift displacement compared to point resolved spectroscopy (PRESS) localization. This is likely valuable in areas prone to inhomogeneities, such as the MTL (Peek et al., 2023).

The reproducibility of HERMES measures in the MTL has not been established. This is particularly key for study using a repeated measures design to track changes over time, such as longitudinal studies of development, neurodegeneration, or treatment studies. Therefore, aim of this study was to 1) quantify the reproducibility of HERMES-sLASER GABA+ (the combined signal of GABA and co-edited macromolecules) and GSH measures in the left MTL, 2) determine whether there are differences in these metabolites between the left and right side in a young, healthy adult population, and 3) provide recommendations for sample size requirements when using this technique.

2. Methods

2.1. Participants

Healthy adults including both males and females, without any contraindications to MRI were recruited to attend a single study visit including MRS. The University of Calgary Ethics Board approved this study (REB23–1157).

2.2. MRS data acquisition

MRS data were collected on a 3 T GE SIGNA UHP MR scanner using a 32-channel head coil. The data acquisition protocol was as follows: Participants were positioned in the scanner, a localizer was run and then a T_1-w anatomical image was acquired (TR/TE = 6.71/2.75 ms, voxel size = 0.43 ×0.43 ×1 mm, flip angle = 10°, phase acceleration = 2, slice acceleration = 1.5, ARC GE deep learning strength = medium) that was used to place the MRS voxel in the left MTL. The voxel was placed on the axial slice and rotated to the angle of the middle cerebral artery before being aligned with the angle of the temporal lobe in the sagittal plane. The voxel was centred to capture as much grey matter as possible. A HERMES acquisition was acquired (TR/TE = 2000/82 ms, 25 ×40 x 25 mm voxel with the 40 mm edge in the anterior-posterior direction, 20-ms editing pulses applied in a Hadamard editing scheme: GABA+ editing pulse = 1.9 ppm, GSH editing pulse = 4.56 ppm; 320 averages, sLASER localization, CHESS water suppression). Participants were then removed from the scanner and repositioned for a second acquisition using the same scanning protocol (localizer, T_1-w anatomical, left MTL HERMES). After this, a third HERMES acquisition was made in the right MTL without repositioning the participant.

2.3. MRS data processing

Data were processed using a custom version of the Osprey software toolkit (Oeltzschner et al., 2020), using MATLAB 2023b and a sequence-specific basis set. The basis set was created using 2D high spatial resolution (101 × 101 points) density-matrix numerical simulations performed with MRSCloud (Hui et al., 2022) and a 1D projection method and coherence pathway filtering to reduce computation time. HERMES data were processed using the Osprey analysis pipeline, including preprocessing, fitting and metabolite quantification. Data were visually inspected for quality assurance. Each MRS voxel was registered to the T_1-w anatomical image used for its localization, and tissue segmentation was completed using SPM12 (Ashburner and Friston, 2005). GABA+ values were tissue- and alpha-corrected (Gasparovic et al., 2006; Harris et al., 2015). The alpha correction adjusts for GABA+ levels being twice that in grey than in white matter (Harris et al., 2015). GSH values were tissue-corrected (Gasparovic et al., 2006). The tissue corrected total creatine (tCr) and N-Acetylaspartate (tNAA) were quantified for each acquisition from the sum spectrum and are presented for completeness. In addition to quantification relative to water, confirmatory analyses using creatine referencing for GABA+ and GSH were performed.

The creatine signal-to-noise ratio (SNR), creatine full-width at half-maximum (FWHM), water FWHM, and relative residuals of each difference spectrum were exported for quality assessment and comparisons.

2.4. Voxel placement analysis

To assess the reproducibility of voxel placement for the two left HERMES acquisitions, the second T_1-w anatomical image was registered to the first T_1-w image using FSL's FLIRT (Greve and Fischl, 2009; Jenkinson et al., 2002; Jenkinson and Smith, 2001). The transformation matrix of this registration was applied to the second left HERMES voxel to visualize visual overlap and calculate a Dice coefficient (Bai et al., 2017; Dice, 1945).

2.5. Statistical analyses

Analyses were performed using SPSS (v29) with figures generated using Python (matplotlib, seaborn, stats models, raincloud plots; Allen et al., 2019; Hunter, 2007; Seabold and Perktold, 2010; Waskom, 2021). Outliers were identified using the interquartile range (IQR), with the threshold set at values more or less than three times the interquartile range from either the upper or lower quartile, respectively. The significance level was set at 0.05. The normality of metabolite values was tested with the Shapiro-Wilk test, and log transformation was used to transform non-normal distributions of metabolite values.

A repeated-measures analysis of variance (ANOVA) was used to investigate differences between the three acquisitions. Post-hoc paired-samples *t*-tests were used to investigate similarity/differences in metabolite concentration (GABA+, GSH, tCr and tNAA), tissue composition, and data quality between the two left MTL voxel measurements and between the right and each of the left MTL measures.

To quantify the reproducibility of the HERMES GABA+ and GSH measures in the left MTL, Pearson's *r* correlation coefficient, the coefficient of variation (standard deviation of the metabolite measures / mean of the metabolite measures) and a difference score of the log-transformed values (metabolite from acquisition 1 – metabolite from acquisition 2) were calculated. The difference score was calculated for each participant separately for GABA+ and GSH. The average difference provides a measure of the within-participant variation and the coefficient of variation quantifies metabolite concentration reproducibility. Additionally, test-retest reliability was assessed with Bland-Altman plots.

To determine if the voxel position affected metabolite variability, the correlation between the Dice coefficient and the coefficient of variation for each metabolite was assessed (i.e., Dice coefficient x GABA+ coefficient of variation and Dice coefficient x GSH coefficient of variation). A correlation would suggest variation in voxel position accounted for variation in metabolite levels.

Finally, the number of participants needed to detect group differences was determined as per (Mikkelsen et al., 2018; Sanaei Nezhad

et al., 2020, cf. (Noordzij et al., 2010) + erratum (Noordzij et al., 2010).

$$N = \frac{2\sigma^2(Z_{1-\alpha} + Z_{1-\beta})^2}{diff^2}$$

In which, N is the number of subjects required per group, σ is the coefficient of variation (or standard deviation relative to mean), $Z_{1-\alpha}$ is the Z for alpha (set at 1.96 for an alpha of 0.05), $Z_{1-\beta}$ is the Z for beta, set to 0.8412 for a beta of 0.8 and *diff* is the difference in means between groups.

3. Results

3.1. Participants

Data were collected from 15 participants (3 males, 12 females) with an average age of 29.5 years (SD = 11.4, range 18–59 years). All participants were right-handed. Data from the right hippocampus were not collected in one participant due to time constraints.

3.2. Quality and normality assessments

One acquisition from the second left MTL had a poor fit of GSH on visual inspection (no visible GSH peak on the spectrum) and was excluded from relevant analyses of GSH. This data point did not meet IQR definition of outliers. However, visually the GSH peak could not be discriminated and it was therefore excluded. Two acquisitions from the right MTL were excluded for all metabolite measures: one due to shim failure resulting in poor data quality and one due to poor fit of both GABA+ and GSH.

Fig. 2 shows all the included spectra, and Fig. 3 shows plots of the GABA+ and GSH values from each of the left and right acquisitions. The Shapiro-Wilk tests were significant for GABA+ in the first left acquisition ($W(15) = 0.87$, $p = 0.038$) and for GSH ($W(14) = 0.87$, $p = 0.044$) in the second left acquisition, so all GABA+ and GSH values were log-transformed for parametric analyses and difference calculations.

3.3. Acquisition differences

When comparing the three repeated acquisitions, a repeated-measures ANOVA showed no significant differences between the log-transformed water-referenced GABA+ measurements ($F(2,22) = 0.26$, $p = 0.78$) or GSH measurements ($F(2,20) = 1.54$, $p = 0.24$). Table 1 summarizes the GABA+ and GSH values, tissue composition and quality metrics from each of the acquisitions, and reports paired sample *t*-tests comparing (a) the left acquisitions to each other, and (b) each left acquisition to the right acquisition. All valid pairs of data were used for the paired *t*-tests.

In the test-retest analysis investigating the two left-sided acquisitions, there were no significant differences in GABA+ or GSH concentration, signal quality metrics or tissue composition (Table 1). There was a small but significant difference in creatine concentration between the two left spectra (second acquisition was 5 % higher, p = 0.017).

There was no significant difference in GABA+ or GSH between the right and left MTL voxel data (Table 1). There was a significant difference between the creatine signal-to-noise ratio and the relative residuals for the GSH spectrum for both left/right comparisons, with the right side having significantly higher creatine SNR and higher relative residuals for GSH compared to both left-sided acquisitions.

There were no significant differences between GABA+ or GSH referenced to creatine in the test-retest analysis of the left side or when comparing GABA+ between the right MTL and each of the left MTL acquisitions.

3.4. Measures of variation

Neither GABA+ ($r(15) = 0.19$, $p = 0.50$) nor GSH ($r(14) = 0.36$, $p = 0.21$) showed a significant correlation between the first and second acquisition. However, the mean differences of the log-transformed metabolite values were calculated to be 0.036 (SD = 0.11) for GABA+ and 0.0001 (SD = 0.15) for GSH, shown in Fig. 4A.

The average coefficient of variation was calculated using the non-transformed values from the two measurements from the left MTL for GABA+ (N = 15) was 14.09 % (SD = 11.10) and the average coefficient of variation for GSH (N = 14) was 18.94 % (SD = 13.06), shown in Fig. 4B.

3.5. Bias plots

The Bland-Altman plots showed no differences and no biases between the first and second left acquisition for either GABA+ or GSH, Fig. 5, indicating these measurements are reliable.

3.6. Voxel position analysis

The average Dice coefficient of the voxel overlap two left voxels was 0.82 (SD = 0.10). There was no correlation between the Dice coefficient and either the GABA+ coefficient of variation ($r(15) = 0.22$, $p = 0.42$) or GSH coefficient of variation ($r(14) = -0.39$, $p = 0.17$).

3.7. Power calculations

The number of participants required to detect 10–30 % group

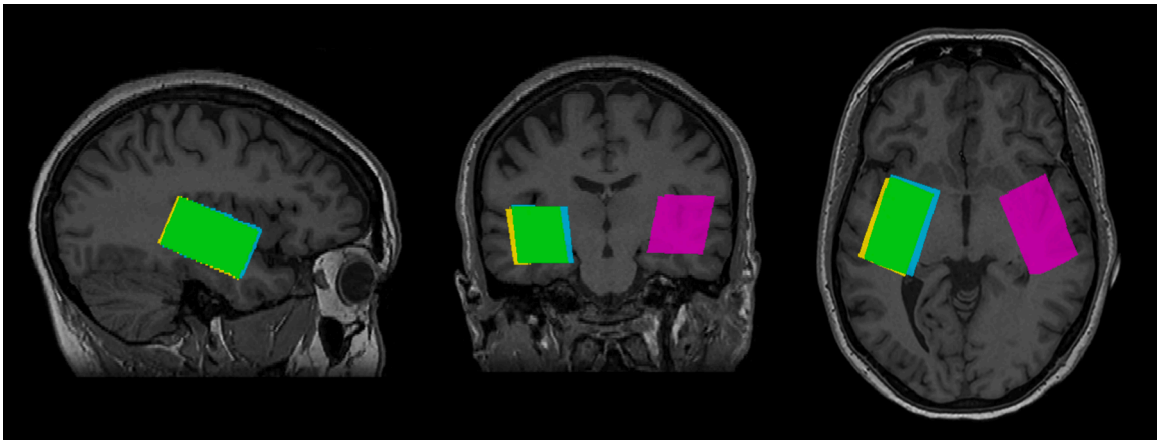

Fig. 1. Example voxel placements and overlap. The two individual left voxels are shown in yellow and blue, with overlap shown in green. The right voxel is shown in pink.

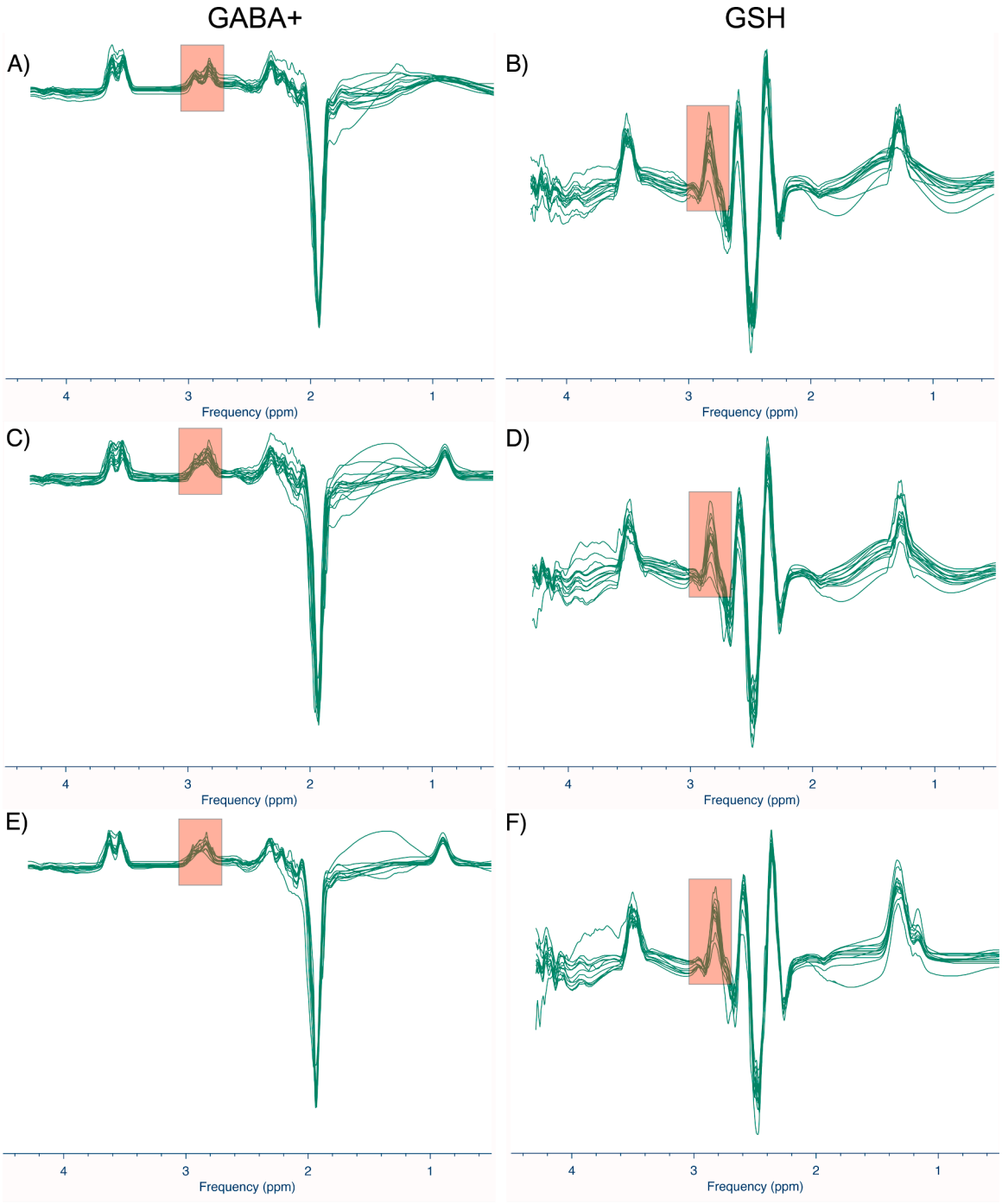

Fig. 2. Difference spectra for all included participants, with red boxes highlighting the area of metabolite peak. A) GABA first left acquisition, B) GSH first left acquisition, C) GABA second left acquisition, D) GSH second left acquisition, E) GABA+ for right side acquisition, F) GSH for right side acquisition.

differences are reported in Table 2 and Fig. 6.

4. Discussion

This study demonstrates the reproducibility of GABA+ and GSH measurements from HERMES-sLASER in the MTL. The coefficients of variation for GABA+ and GSH were 14.09 % and 18.94 %, respectively, and comparable to metabolite specific edited-MRS measurements of these metabolites in this MTL region (Bednarik et al., 2015; Vidyasagar et al., 2024; Volzke et al., 2021). A study using MEGA-sLASER at 7 T to measure GABA+ in the hippocampus obtained a CV of 12.3 % (Volzke et al., 2021), slightly lower but comparable to the 14.09 % for GABA+ found here. For GSH, an unedited sLASER measure using a 4 mL voxel in the hippocampus reported the coefficient of variation of GSH is < 20 % (Bednarik et al., 2015). An study using GSH-edited MEGA--PRESS in the MTL, using a similar voxel to the present study, found a coefficient of variation of 21.97 % (Vidyasagar et al., 2024). The current study used sLASER localization, and its reduced chemical shift displacement may have improved the reproducibility of GSH measurements.

In addition to comparing results to GABA+ and GSH studies of the MTL, it is relevant to compare the current study with HERMES studies in other brain regions to inform unique challenges in the MTL. To our knowledge, there has only been one other HERMES reproducibility

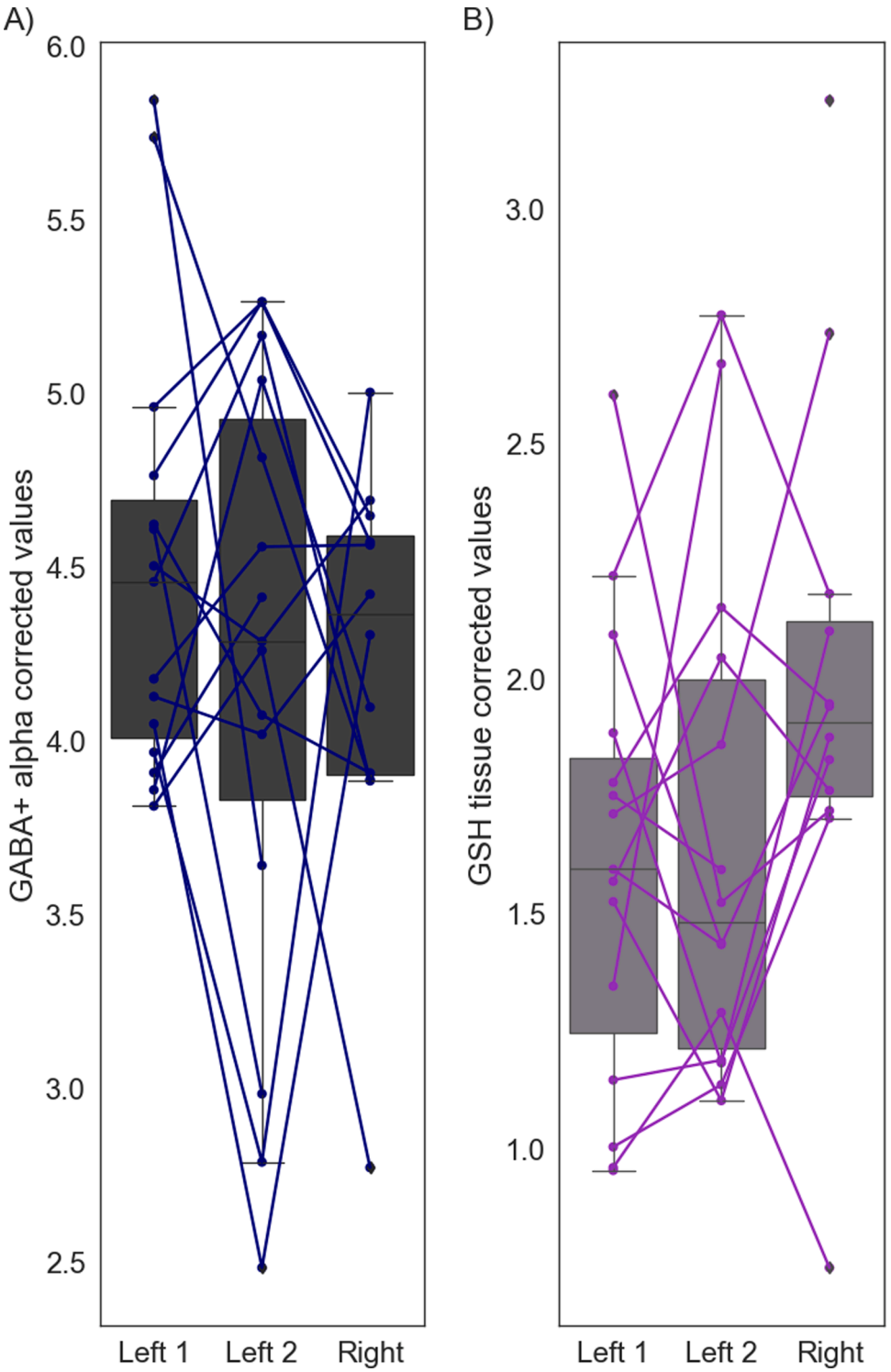

Fig. 3. GABA+ alpha-, tissue-corrected values, and GSH tissue-corrected values from both left and right acquisitions. Each point represents one acquisition, with lines joining participants across acquisitions. Boxes represent the interquartile range, and the whiskers extending to the most extreme value within 1.5 times the interquartile range from the hinge, as per Seaborn norms.

study, which examined a voxel in the dorsal anterior cingulate cortex and used PRESS localization (Prisciandaro et al., 2020). Prisciandaro et al. (2020) found coefficients of variation were 16.7 % for GABA+ and 19.0 % for GSH, again comparable to the coefficients of variation in the current study. Prisciandaro et al. (2020) also found a notably lower coefficient of variation for GSH (7.3 %) when using a specific GSH-editing sequence with an echo time of 120 ms which is specific to GSH. They therefore suggest that if GSH is the key outcome of interest then a specific GSH-edited sequence may be preferable. However, Vidyasagar et al. (2024) used an echo time of 130 ms in their GSH-edited data of the MTL and did not show a similar benefit to the coefficient of variation. As the optimization of GSH-editing is beyond the scope of this reproducibility study, the advantages of a longer echo time and how this interacts with sLASER localization and the region of interest remain an outstanding question.

This study found no correlations of GABA+ or GSH between the two

Table 1
Voxel metabolite values, tissue composition and quality metrics. tCr: total creatine, fGM: Fraction of gray matter in voxel, fWM: Fraction of white matter in voxel, fCSF: Fraction of cerebrospinal fluid in voxel, SNR: Signal-to-noise ratio, FWHM: Full-width at half-maximum, tNAA: Total *N*-Acetylaspartate. The relative residuals are the relative amplitude of the residual of the respective metabolite over the standard deviation of noise. *The parametric analyses of these values were conducted on the log-transformed values, while the raw values are reported here.

Variable	Left 1 M(SD)	Left 2 M(SD)	Right 1 M(SD)	Paired t Left 1 vs Left 2	Paired t Left 1 vs Right	Paired t Left 2 vs Right
GABA+ (alpha corrected)*	4.49 (0.63)	4.20 (0.9)	4.23 (0.58)	$t(14) = 1.28$, $p = 0.22$	$t(11) = 0.91$, $p = 0.38$	$t(11) = 0.11$, $p = 0.91$
GSH (tissue corrected)*	1.61 (0.48)	1.67 (0.55)	1.98 (0.6)	$t(13) = 0.001$, $p = 0.999$	$t(11) = -1.87$, $p = 0.09$	$t(10) = -1.41$, $p = 0.19$
GABA+ /tCr*	0.76 (0.11)	0.69 (0.14)	0.71 (0.09)	$t(14) = 1.73$, $p = 0.11$	$t(11) = 1.34$, $p = 0.20$	$t(11) = -0.18$, $p = 0.86$
GSH/tCr*	0.26 (0.07)	0.25 (0.08)	0.31 (0.08)	$t(13) = 0.51$, $p = 0.62$	$t(11) = -2.11$, $p = 0.059$	$t(10) = -2.01$, $p = 0.072$
fGM	0.51 (0.03)	0.51 (0.03)	0.53 (0.03)	$t(14) = 0.581$, p = 0.57	$t(11) = -1.691$, $p = 0.119$	$t(11) = -1.558$, $p = 0.148$
fWM	0.42 (0.04)	0.43 (0.04)	0.4 (0.05)	$t(14) = -0.408$, $p = 0.69$	$t(11) = 1.391$, $p = 0.192$	$t(11) = 1.432$, $p = 0.18$
fCSF	0.06 (0.02)	0.06 (0.01)	0.07 (0.02)	$t(14) = -0.025$, $p = 0.981$	$t(11) = -0.494$, $p = 0.631$	$t(11) = -0.751$, $p = 0.469$
Creatine SNR	113.19 (16.22)	116.92 (19.31)	137.28 (19.06)	$t(14) = -0.889$, $p = 0.389$	*$t(11) = -4.513$, $p = <.001$*	*$t(11) = -2.207$, $p = 0.05$*
Creatine FWHM	10.77 (1.72)	11.21 (2.82)	9.54 (1.66)	$t(14) = -0.828$, $p = 0.422$	$t(11) = 1.651$, $p = 0.127$	$t(11) = 0.688$, $p = 0.506$
Water FWHM	11.29 (1.31)	11.43 (2.16)	10.31 (1.39)	$t(14) = -0.355$, $p = 0.728$	$t(11) = 1.61$, $p = 0.136$	$t(11) = 0.414$, $p = 0.687$
Relative residual GABA+ spectrum	3.15 (1.2)	3.15 (1.28)	3.28 (0.88)	$t(14) = -0.008$, $p = 0.994$	t(11) = −1.053, p = 0.315	t(11) = −0.08, p = 0.937
Relative residual GSH spectrum	2.08 (1.47)	1.94 (1.36)	1.86 (0.31)	t(13) = 0.4, p = 0.695	*$t(11) = -2.809$, $p = 0.017$*	*$t(10) = -2.327$, $p = 0.042$*
tCr from sum spectrum	6.25 (0.42)	6.55 (0.55)	6.29 (0.57)	*$t(14) = -2.711$, $p = 0.017$*	$t(11) = -0.45$, $p = 0.661$	$t(11) = 1.292$, $p = 0.223$
tNAA from sum spectrum	9.57 (0.85)	9.8 (0.91)	9.84 (0.88)	$t(14) = -0.881$, $p = 0.393$	$t(11) = -0.544$, $p = 0.597$	$t(11) = 0.137$, $p = 0.894$

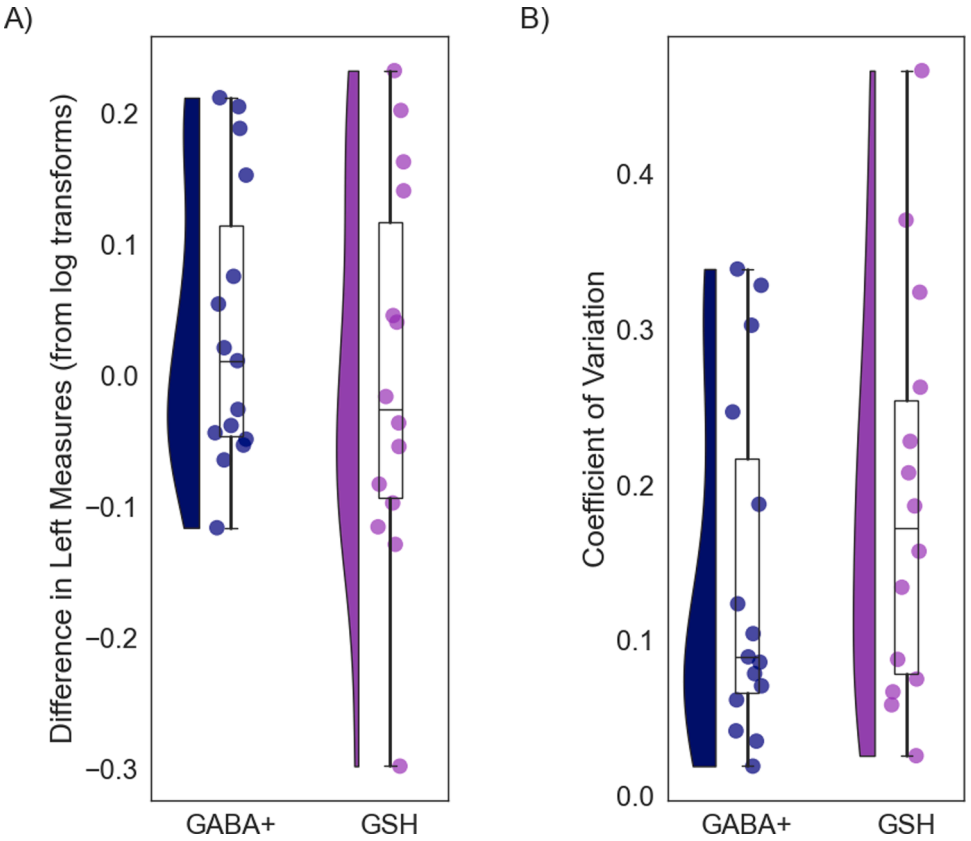

Fig. 4. A) Individual differences in GABA+ and GSH levels between the first and second left acquisitions after log transformation. B) Individual's coefficients of variation of GABA+ and GSH for the left side acquisition. Each dot represents a value from a participant. Boxes represent the interquartile range, and the whiskers extending to the most extreme value within 1.5 times the interquartile range from the hinge, as per Seaborn norms.

repeated left-sided acquisitions. The difference between these two measures, however, was centred on zero. This suggests that in this small sample with little variability in metabolites, noise results in the inability to detect a test-retest correlation. When investigating lateral differences, there were no differences in metabolite values between the left and right MTLs. While this result supports the reliability of HERMES-sLASER to examine group differences, longitudinal analyses with small changes over time may be challenging.

One factor that can impact measurement reproducibility in single voxel MRS is the reproducibility of voxel placement and, therefore, the tissue included in the measurement. It is established that brain metabolite concentrations in the cortex are variable (DeMayo et al., 2023), and there is no reason to expect it to be different in subcortical structures. The large voxel is unavoidable when performing edited-MRS, but our voxel placement showed good reproducibility, especially given the participant got out between the scans (Bai et al., 2017). With a 1 mm translation in each direction, with our voxel dimensions, the Dice coefficient would be 0.89, thus a Dice of 0.82 including rotations and

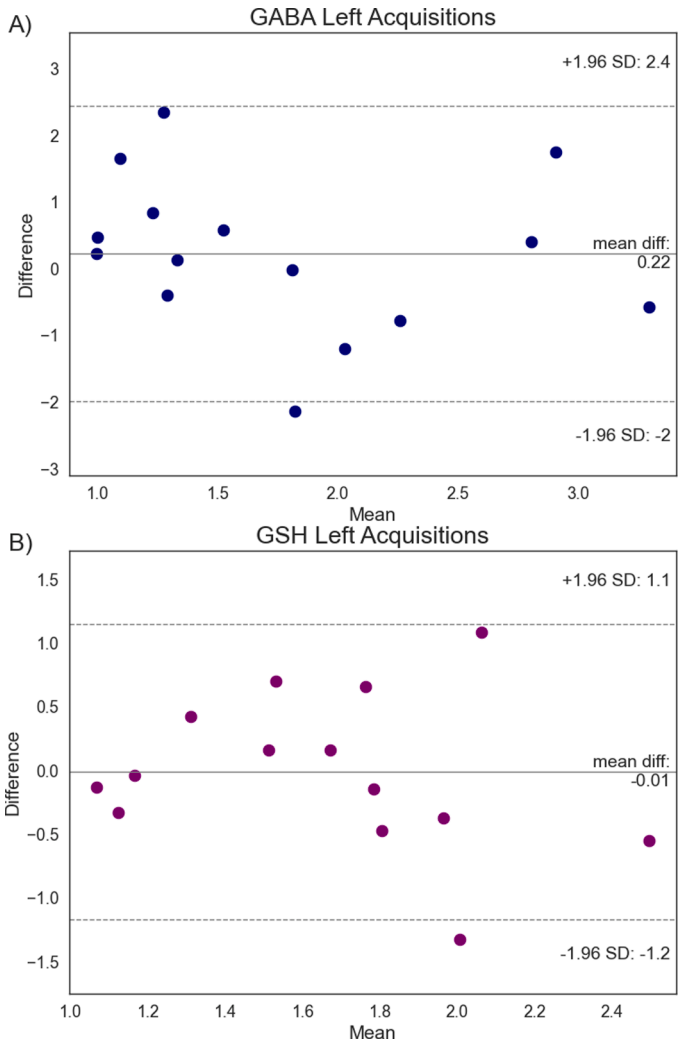

Fig. 5. Bland-Altman plots for GABA+ (top panel) and GSH (bottom panel). The mean of the metabolite left acquisitions is shown on the x axis and the difference between the two acquisitions on the y-axis, for GABA+ and GSH.

Table 2
Numbers required to detect group differences of 10–30 % for GABA+ and GSH in the MTL.

Percent Difference	GABA+	GSH
10	32	57
15	14	25
20	8	15
25	5	9
30	4	7

translations for registration demonstrates strong voxel placement reliability. The Dice coefficient was not associated with coefficients of variation, suggesting that differences in voxel position did not contribute to intra-individual variance and that subtle differences in captured tissue should not significantly alter findings.

There were differences between acquisitions in quality measures and metabolites quantified from the sum spectrum. There was a small but significantly higher amount of total creatine quantified in the second left acquisition compared to the first (4.6 % higher, $p = 0.02$). The source of this difference is unclear, particularly given the non-significant effects on SNR and linewidth. Still, given the size of this difference, we suggest that it is non-meaningful. The relative residuals of the GSH spectrum (the signal of the GSH peak referenced to the standard deviation of the noise) and the signal-to-noise ratio of creatine were significantly different in the acquisition from the right side compared to both left-sided acquisitions. While there may be some trends in creatine data quality, broadly, the data is of high quality, which is the biggest concern for edited single voxel spectroscopy in the MTL. Further, these differences are small, so while significant, they don't appear meaningful.

The coefficients of variation from reproducibility studies such as this one can be used to assist in the calculation of cross-sectional/group-based sample sizes for future research, especially given the limited HERMES data otherwise available in the MTL and hippocampus. We find the required sample size to detect group differences in GABA+ are comparable to those for using MEGA-PRESS reported in Mikkelsen et al. (2018) where they reported that for a 15 % difference in GABA+, groups of 14 are needed. In our study, given the greater variability in GSH, we suggest 25 participants are required to detect a 15 % difference in GSH levels. While these sample size calculations can support design future study design, it is relevant that this was a healthy adult population and likely has intrinsically low variability. In, for example, a clinical population, the effects of lower and/or more heterogeneous metabolite levels combined with tissue atrophy and possibly lower data quality may increase the variability of measures and thus increase the required sample sizes.

5. Conclusion

This study suggests HERMES-sLASER can be used in the MTL to quantify GABA+ and GSH. The measurement of these metabolites evidenced comparable reliability to HERMES data acquired in cortical brain regions and to standalone measures of these metabolites within the MTL. Finally, it provides guidance on participant numbers required to see group differences in the MTL.

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CRediT authorship contribution statement

Marilena M. DeMayo: Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Data curation, Conceptualization. **Mary Botros:** Writing – review & editing, Project administration, Methodology, Formal analysis, Data curation. **Tiffany K. Bell:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Mark Mikkelsen:** Writing – review & editing, Software, Methodology, Formal analysis. **Victoria Mosher:** Writing – review & editing, Resources, Project administration. **Antis George:** Writing – review & editing, Resources, Project administration. **Alexander McGirr:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Paolo Federico:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Ashley D. Harris:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests Ashley D Harris reports financial support was provided by Natural Sciences and Engineering Research Council of Canada. Marilena M DeMayo reports financial support was provided by Canadian Institutes of Health Research. Tiffany K Bell reports financial support was provided by

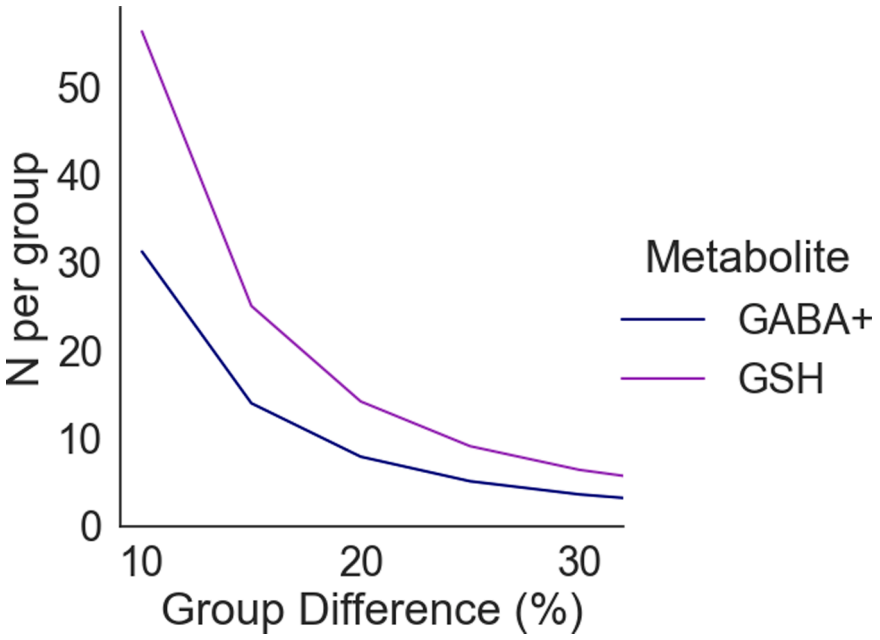

Fig. 6. Plot of the number needed within each group to determine various group difference effects.

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Data availability

Data will be made available on request.

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